Behavioral differences between populations of an African cichlid fish from divergent habitats

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Abstract

With all of the different environmental shifts occurring in ecosystems across the globe due to anthropogenic activities, it is not hard to imagine that in order for a species to survive, it needs to adjust its phenotypic traits. In aquatic systems, a number of environmental changes are known to affect the survival of fish, including low dissolved oxygen (hypoxia) and increased suspended sediments (turbidity). *Pseudocrenilabrus multicolor* is a species of African cichlid fish that is widespread across East Africa and is found in a range of habitats that vary in dissolved oxygen (DO) and turbidity level. My study tested the hypothesis that there are persistent differences in reproductive behaviors across populations due to the environmental differences the fish experience in their natural habitats (swamp = low DO, clear; river = high DO, turbid). To test this, I made repeated observations of F1 fish from four populations (two swamp and two river populations) reared in the lab under common garden, low stress conditions (high dissolved oxygen, low turbidity). Each of 20 aquaria (5 per population), holding one male and two females, were observed for ten minutes (each week for five weeks) during which I recorded any behavior demonstrated by the fish. The behaviors were divided into two categories, reproductive behaviors and aggressive behaviors, and the mean ratio of reproductive to aggressive behaviors calculated per aquarium. Two separate one-way ANOVAs indicated that there were no behavioral differences among the four populations, or between two habitat types when populations were pooled. This could indicate that the effort placed into reproductive behaviors is flexible, and that there is a strong plastic component to the behavior.

Introduction

Reproductive behaviors are a key component to the survival and success of many species on Earth. Organisms need to reproduce in order to continue the validity and presence of their species in their ecosystem. It is important for reproduction to be recombinant because a diverse genetic make-up in a species allows for greater future persistence of the population. Animals need to allocate their energy towards growing and reproducing, and there often is a trade-off that develops between the two processes that changes over developmental time. Across ontogeny, we would expect to see differences in the way animals allocate their energy based on their state (Candolin and Heuschele, 2008). Reproduction has long been studied as not only a way a species ensures its survival, but also as a way it continues to adapt. Animals will often use species-specific behavioral displays to attract a mate when in a reproductive state. However, if the environment changes, the animal could then change how they attract a mate. An individual could allocate energy into different things, such as foraging or fighting for resources (Candolin and Heuschele, 2008). The study of reproduction has allowed scientist to biologically and socially engulf themselves into a species and how they function in the wild.

Two forms of evolutionary processes allow for species to behave the way they do in order to ensure survival. The first being natural selection, a concept introduced by Charles Darwin in 1859. Natural selection is the preservation of favorable traits and the rejection of useless or fatal variations (Darwin, 1859). Natural selection favors traits that increase survival, leading to a future generation better adapted to the environment. Darwin also defined the second evolutionary pressure as sexual selection, in 1871 (Trivers, 1972). Sexual selection is the competition between one sex of a species for access to members of the opposite sex, as well as the differential choice by members of one sex for individuals of the opposite sex (Trivers, 1972). By investigating these two forms of selection scientists have the ability to determine how and why an animal allocates their energy, and whether they are choosing to spend their time attempting to be reproductively successful, or attempting to survive as an individual. These processes can be disrupted when humans change the environment.

Humans are constantly utilizing natural resources, which drastically alters natural ecosystems. Increased industrialization, urbanization, and intensive agriculture over the past century have resulted in changes, especially to aquatic environments (Van Buskirk, 2012). In regards to aquatic systems, there are a number of factors that affect the survival of an aquatic species. Two important factors are turbidity and dissolved oxygen (Chapman et al., 1996; Crispo and Chapman, 2008, 2010, 2011). Turbidity is the measurement of suspended particles, specifically clay or dirt, in the water. This is measured by how much light can travel through the water, which essentially measures how clear or cloudy the water is (Utne- Palm, 2002). Increased turbidity can result in added stress to aquatic systems, which in turn decreases the diversity of ecosystems across the globe (Ricciardi and Rassmusen, 1999). Turbid waters may decrease the ability of fish to see. Because of changes in the visual environment, there can be disruptions in communication signals, which in some cases has been shown to break down species recognition signals, alter mate choice, and lead to the loss of biodiversity (Seehausen et al., 1997). Fish may also adjust their behavior to become more visible to a mate. For example, male cichlid fishes increased their courtship behaviors in order to compensate for increased turbidity (Gray et al., 2011).

Dissolved oxygen is also important to the survival of fish, and the higher the dissolved oxygen, the greater the conditions (Chapmen et al., 2000). If the dissolved oxygen levels are extremely low, the water is considered to be hypoxic, which makes it hard for fish to get enough oxygen to survive. Again, some fish are able to adapt to stressful environments such as hypoxia. Chapman et al. (2000) found that the African cichlid *Pseudocrenilabrus multicolor victoriae* from hypoxic waters tend to have larger gills and smaller brains, with lower standard metabolic rates (Chapman et al. 2000). *P. multicolor* in low dissolved oxygen environments also show decreased levels of activity, with behavioral flexibility that comes with physiological costs of low levels of oxygen (Chapman and McKenzie, 2009). Males in hypoxic waters are also redder in color than fish in water with higher dissolved oxygen, which is most likely to allow them to attract mates without having to do physical displays that require energy (McNeil et al., 2016). These two environmental conditions can change drastically due to anthropogenic events (Kemp et al., 1998) and result in behavioral shifts in the fish of those waters (Sih et al., 2011).

Behavioral responses from animals inhabiting altered ecosystems can result in changes in species distribution, adaptation, speciation, and their extinction rates, which affects the overall biodiversity of the planet (Tuomainen and Candolin, 2011). Two types of behaviors that are often observed in social fish are aggressive and reproductive behaviors. Aggressive behaviors are typically associated with competition for resources (e.g. food, space). Reproductive behaviors are those displayed, typically by a male, to attract the opposite sex for means of reproduction. These can also be described as courting behaviors or sexual displays. Sexual displays are behaviors exhibited that allow one gender of fish to present to the opposite gender the opportunity to reproduce. This is important to fish because it allows communication between them to indicate the intent to reproduce. It also allows for genetic variation, providing one gender to make selections towards preferences in another gender. In one example, the strength of sexual selection decreases in species such as stickleback (*Gasterosteus aculeatus*) when there are changes in the visual environment (Candolin et al., 2007; Wong et al., 2007). Candolin et al. (2012) expanded on this finding, by completing research to conclude that sticklebacks living in increased turbidity (specifically in the Baltic Sea) spend more time courting females than males in clear water.

Recent studies have shown that increases in turbidity have resulted in behavioral and phenotypic changes in *P. Mulitcolor*. Turbidity can also lead to a decrease in fish diversity, as Seehausen et al. (1997) discovered with an endemic haplochromine cichlid fish found in Lake Victoria in East Africa. A narrowing of the light spectrum due to overly enriched waters decreased light transmission, which limited the usefulness of color signals in cichlid fish, therefore decreasing overall species diversity (Seehausen et al. 1997). In a turbid environment, activity might decrease due to overall stress (Ricciardi and Rassmusen, 1999; Ritcher et al., 1997). Random interactions related to competition become especially risky due to increased predation risk and exhaustion because of increased energy use (Ros et al., 2006; Briffa and Sneddon, 2007). However, we might see an intensification in reproductive behaviors to ensure population success in their ecological niche (Gray et al., 2012), or a trade-off between reproductive behaviors and social behaviors in a stressful, turbid environment (Jones and Reynolds, 1997). Threespine sticklebacks have reduced levels of sexual selection due to changes in their visual environment because of turbidity (Candolin et al. 2007; Wong et al. 2007). The decision to display more or less is influenced by evolutionary

Social behaviors have also been researched, as cichlid species including *P. multicolor* are extremely social. Males defend territories and attract mates using detailed male to male aggression (Fernö, 1986). In low oxygen environments, *P. multicolor* decreases their social behavior levels by behaving less compared to fish from high oxygen streams. This is likely to make up for the costs the fishes physiological systems take in a low dissolved oxygen environment (Chapman and McKenzie, 2009). However, little research has been completed to determine if there are differences in this social behavior, between the different populations. There has also been no research to date completed on if these behaviors observed, are consistent across a population of fish whether they are in their natural environment, or present in a different habitat.

Pseudocrenilabrus multicolor victoriae (Seegers, 1990) is a species of African cichlid fish that lives in freshwater rivers and swamps and is widespread across East Africa (Hanssesns, 2006). *P. multicolor* is a sexually dimorphic species, meaning that the males and females differ in phenotypic traits. The males are significantly larger, and bright orange in coloration (Figure 2), while females are smaller in size, and are dully colored. Male *P. Multicolor* are often more aggressive to each other in turbid waters, rather than in clear waters. Additionally, they react quicker to a stimulus in turbid waters, which is a rapid response due to the fact that behavior can be altered quickly as a result of an environmental stressor (Timmerman and Chapman, 2004). Males in turbid waters also experience increased levels of aggression in comparison to males in less turbid waters of the same species (Gray et al. 2012). Research by Gray et al. (2012) also suggested a plastic component in response to changes in turbidity. A plastic component is a behavior or phenotypic trait that can shift in order to better fit the needs of the organism at that time. This allows *P. multicolor* to intensify reproductive behaviors and decrease general activity in order to increase reproductive success (Gray et al., 2012).

Within the Gray Physiological Ecology Lab, there are four separate populations of adult *P. multicolor* that were caught in Uganda and transported back to The Ohio State University. The first pool being river populations, located in the Bunoga and Ndyabusole rivers. These rivers contain high levels of turbidity, as well as high levels of oxygen in the water. The second pool is freshwater swamp populations that include the Bwera and Lwamunda populations. These swamps have low turbidity and are very clear, however their water has low levels of oxygen, making the water hypoxic (Chapman et al., 1996, 2008, 2010, 2011). A map of these drainages can be seen in Figure 1. *P. multicolor* is a species that has suited this type of research well because of their relatively simple social structure, and sexual dimorphism. They are easy to reproduce in a lab setting, and are easy to take care of in a lab environment, requiring little maintenance compared to other freshwater species. Along with that, they have sexual displays and courting, which allow them to use their elaborate behaviors to be quickly observed in behavioral studies. They are adaptive as the displays may shift to show off different traits, or maybe become larger or more frequent to be noticed more. Finally, *P. multicolor* is a good species to use to understand the impacts that animals and plants face from shift environments, primarily caused by humans and their interactions with the Earth's resources. The environmental changes the fish are experiencing are primarily due to anthropogenic events. The fish then need to make changes in order to survive to our influence on their ecological niche, which is something that not only *P. multicolor* experiences, but animal species around the world as well.

My main goal was to test if *P. multicolor* reproductive behaviors are consistent across all four populations no matter the environmental condition of the parental population. To do this I used fish from four F1 populations that were reared under common garden conditions (i.e. clear, high dissolved oxygen water). Common garden experiments include placing the same species of an organism from different populations and from different ecosystems in the same type of environmental conditions. The specific objectives were to (a) determine if there are behavioral differences between all four populations of *P. multicolor* when reared under common garden conditions, and (b) determine if there are behavioral differences between F1 *P. multicolor* whose parents originated from different oxygen and turbidity conditions based on drainage (i.e. swamp versus river). If reproductive behaviors result from genetic differences between populations, then we would expect to see a difference in behavior between populations. Alternatively, if the behaviors are more plastic, then there would be no difference between the populations when the environmental conditions are the same, and with no stress.

Methods

Fish care and housing

The fish used in this study were reared in the laboratory from wild-caught parents collected in Uganda in the summer of 2016. Adult, lab-reared F1 fish from four populations (Nabugabo Drainage: Lwamunda (swamp), Ndyabusole (river); Mpanga Drainage: Bwera (swamp), Bunoga (river)) (Figure 1) were distributed among 20 aquaria in the Kottman Hall Greenhouse. The fish were fed every day a diet of Tetramin flake food. Temperature was maintained at approximately 25ºC. Once a week more extensive fish care was completed, including water quality testing for nitrite and ammonia, temperature, dissolved oxygen, and conductivity. A minimum of a 30% water change was completed during these weekly cleanings. All of the data was recorded in a notebook and binder located outside in the greenhouse as per IACUC protocol #2014A00000055-R1-AR1.

In each of the tanks, I placed one male and two female fish, all three coming from the same population. A picture of example male and female *P. Multicolor* can be seen in Figure 2. All aquaria were held under the same common garden environmental conditions that were used for rearing: all twenty tanks contained freshwater, with high dissolved oxygen levels (~8 mg/L), and low levels of turbidity (<1.0 NTU, as the water was completely clear. These considered are ideal conditions, as they are low stress and leave the fish in a comfortable environment. Each aquarium also contained one artificial plant and a small layer of sand at the bottom. The plant allowed for hiding behaviors and the sand allowed for burrowing and digging behaviors, which can be part of a courting or just exhibited by males wishing to mate. The sand was added, followed by treated water that we keep in vats in the greenhouse, followed by adding the air supply, and a plant to finish. The tanks were allowed 24 hours to circulate before the fish were added. I placed black, plastic barriers between aquaria, so that they were isolated and could not see each other.

Behavioral Observations

A behavioral observation study was completed to determine the proportion of reproductive relative to social behaviors displayed by each population. Fish were separated based on population into 5 aquaria per population (total aquaria = 20). Each aquarium held approximately 9 liters of treated water.

The tanks were observed for ten minutes at a time by a single observer. Five observations were made for each of the aquaria, resulting in 50 minutes of observation per tank, and a total of 250 minutes of observations per population. Observations were made once a week, typically in the mid to late afternoon hours (i.e. after 12:00PM EST). The courting behaviors observed were divided into two categories, aggressive behaviors and reproductive behaviors (Table 1). Aggressive behaviors included bites, which was when a male or female used their mouth to attempt to puncture a fish of the opposite sex, and chases, which consisted of a male chasing a female around in the tank. The reproductive behaviors were leads, quivers, lateral displays, and frontal displays. Quivers consist of the male fish shaking in place (typically over a sand pit that he has dug), while leads require the male to swim from the female to the sand, in order to imply that he wishes to mate. Frontal displays are when the male fish presents himself to the front of the female fish, while lateral displays require the male to present himself to the side of the female.

Once observations were completed, the mean reproductive and mean aggressive behaviors were calculated for each of the twenty tanks across their five observations. The ratio of reproductive behaviors to aggressive behaviors was then calculated for each tank. This was done so that we could test if populations or habitat types differ with respect to the amount of effort they devote to reproductive versus aggressive behaviors. This ratio indicates to us how frequently the male fish being observed is presenting an aggressive or courting behavior. We then performed two one- way ANOVAs. In the first ANOVA, we compared the ratio between reproductive and aggressive behaviors among the four populations. The second ANOVA compared the ratio between reproductive and aggressive behaviors between the habitat types, two swamp populations pooled versus the two river populations pooled.

Results

We detected significant differences in the variances between populations (Levine's test for homogeneity of variances $P < 0.05$). A log transformation was completed on all of the mean proportions, and one-way ANOVA'S for the four populations and two habitat types were performed using the transformed data. This log transformation was completed to improve the homogeneity of the variances by making the highly skewed data less skewed. ANOVA assumes that the variances are equal among populations. Therefore, the dependent variable was the log reproductive: aggression ratio and the independent variable was the population or the habitat type.

The one-way ANOVA testing for behavioral differences between the four populations observed revealed there was no significant difference between the ratio of reproductive to aggressive behaviors ($F_{3,16} = 0.700$, $P = 0.566$; Figure 3A). Additionally, there was no significant difference in behaviors between pooled swamp vs. river populations ($F_{1,18} = 0.394$, $P = 0.538$; Figure 3B). No matter how the data is compared against each other, there is no significant difference between the populations or habitat types, and what their reproductive behaviors are.

Discussion

Although no significant differences were found in the ratio of reproductive to aggressive behaviors among the different populations or their habitats, the results provide us with new information that can help us better test future predictions. Our lack of a difference could imply that behaviors that are exhibited in turbid versus clear waters, such as increased aggression, can be due to an acclimation the fish has acquired to adjust their behavior only according to the present surroundings. If their reproductive behaviors were linked genetically, these changes would happen regardless of the environment they were placed in.

We are unable to determine if the fish's reproductive and courting behaviors are developed as a genetic trait, an acclimation, or as a learned trait. If the trait is genetic, it could be that the changes are happening so rapidly to their environment, that in order to survive the fish must acclimate their behaviors (Wong and Candolin, 2015). Evolution takes a significant amount of time, so the behaviors may not have undergone a transformation into a genotypic adaptation as the environment continues to shift (Candolin et al., 2008). Research has shown that many behavioral responses due to anthropogenic events are on a case by case base, or an acclimation to the environment they are in (Barrett and Hendry, 2012). There have only been a few noted cases of population-level evolutionary responses, which unfortunately includes the broad definition of the evolution of behavior (Merilä and Hendry, 2014). This limitation in the approach can be seen only in situations where phenotypes of individuals can be tracked across the span of multiple events (Merilä and Hendry, 2014). In order for a mutation to occur, time is needed, as well as an increase in the changed behavior occurring to emphasize the increase of importance of the behavior (Futuyma, 2010). Limited genetic variation could be the reason that this process does not occur sooner, which forces the gene to wait for gene flow or mutations to take over and provide this variation to the population (Wong and Candolin, 2015). An example of this is outlined in Wong and Candolin 2015, as an individual organism during mating that is bold could receive a benefit from being shy during foraging to avoid a predator. There can sometimes be genetic correlations between behaviors that can cause constrains on adaptations (Wong and Candolin, 2015).

If the behaviors are not genetic, this suggests a plastic component to their reproductive behaviors. There is a detailed amount of research suggesting that plastic behavioral responses are often adaptive (Wong and Candolin, 2015). However, many behavioral responses can be maladaptive as well, or acclimated to their situation at hand. Environmental changes can result in mix up between the cues provided by the environment, and the actually quality of the habitat, resulting in an "ecological trap" (Schlaepfer et al., 2002; Robertson et al., 2013). Ecological traps occur when an organism makes unfavorable habitat choices because of cues that were previously related to their habitat quality before being altered by humans (Schlaepfer et al., 2002). In *P. multicolor,* this is seen in fish under low dissolved oxygen environments, as it is suggested that reproductive displays under these conditions start to decrease over time, as they require high amounts of energy to display, which may not be available in this kind of environment (Gotanda et al., 2011). These maladaptive traits can either be a learned trait or acclimated trait. A learned trait can be picked up from other fish that are completing the same task successfully. If a fish struggling to reproduce in turbid waters observes a fish complete the task successfully, then they are able to learn how to properly court in an environment where vision is poor. On the other hand, this method of courting in changing environments could be something the fish are learning as they go along. It is habituated to make adjustments to the way they act in order to compensate for the change in their habitat. Both of these types of acclimations are plastic amongst the fish, as it can shift based on when the trait is needed. All the same, animals may not be able to adjust quick enough to the anthropogenic effects to counter the rapid changes (Van Buskirk, 2012). Futhermore, events such as pollution have been found to disrupt sensory cues, for example in swordtail fish and their olfactory cues (Fisher et al., 2006) *Xiphophorus birchmanni* use chemically mediated signals for recognition, and these can be effected due to anthropogenic disturbances that effect the environment in which they send the signal through (in this case, freshwater) (Fisher et al., 2006). When exposed to high levels of humic acid in their freshwater environment, female swordtail fish lost their preferences for conspecific male chemical cues (Fisher et al., 2006). This leads to a decrease in biodiversity and can cause a decline in species isolating mechanisms (Fisher et al., 2006).

Similar situations have been observed in other species both aquaticand terrestrial. Extreme noise exposure is a stressor added to natural environments by humans that has notable effects on animal behavior (Wright et al., 2007). Not only can the short term exposure to the sound cause long term consequences in behavior as a primary stressor, but the overall annoyance of the sound can cause a secondary stressor of irritation to the animal (Wright et al., 2007). In marine mammals, anthropogenic sounds such as military sonars, energy development, and offshore construction alter not only the habitats, but masks the communication of the species (Ellison et al., 2011). These signals are important for the marine species in the water to communicate for mating, feeding, navigation, and to avoid predators (Ellison et al., 2011). Similarly, in song birds such as the *Sturnus unicolor* and *Passer domesticus,* noise from human activity cause a shift in the timing of bird song activity (Arroyo- Solis et al., 2013). Dawn-singing species like the spotless starling and house sparrow start singing earlier in the morning in areas with high levels of noise and high base-line amplitude levels in Seville, Spain (Arroyo-Solis et al., 2013).

The skewed data can be potentially explained by the vast possibility for error during a behavioral study. For example, male and female variance is common when courting, as males are not always successful and females are not always interested. The time of day the observation was made, as well as the time of day they were fed were inconsistent throughout the study, which could also explain some variance. Females often behave less once they are brooding, so any lack of behavior could have potentially been recorded as zeros, but was really due to brooding. Continued statistical analysis can be completed on future projects to account for the potential of error.

Further research and data collection can be completed to explain how behaviors can alter in an environment under a common garden. Longer and more detailed observations can be done to result in a greater breath of information about the population's behaviors. These can be in a common garden experiment, to continue to test for a genetic link, or in an environment similar to that of their wild population, to test if there is a difference present only when the environment changes. A larger selection of behaviors can be added to the observations, such as courtship behaviors, and non- reproductive behaviors that are included in their daily survival.

To test if there is a genetic component to the behavior, a behavioral study similar to this one can be completed, followed by the rearing of a F2 generation. Behavioral observations in a common garden set up can be completed on the F2population to see if the males exhibit the same traits when courting that their parent did. Hybrid breeding can also be used to see if a mix between F2 populations is present in courting behaviors, further indicating that their courting behavior is trait that is passed down to offspring.

An increased sample size would also benefit this experiment, as a greater number of observations and tanks would create a less skewed data pool with more numbers to contribute to the analysis. More than five observations on a tank would also increase the success of future studies, as the fish inside of the tanks would be able to develop mutual relationships towards each other, which could decrease female mortality, and increase reproductive success.

The environment in which a *P. multicolor* lives effects the behavior of that individual cichlid fish. However, the reason for shifts in their behavior arises from a changing environment that increases stressors on only those individuals who experience it. Through further research adding to the already extensive depth of information we have recovered, a direct cause and effect of these changing cichlid behaviors can be determined, and the information can be used to allow us to help the fish adapt as the environment around them shifts.

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Tables

Table 1: Behaviors recorded during observations

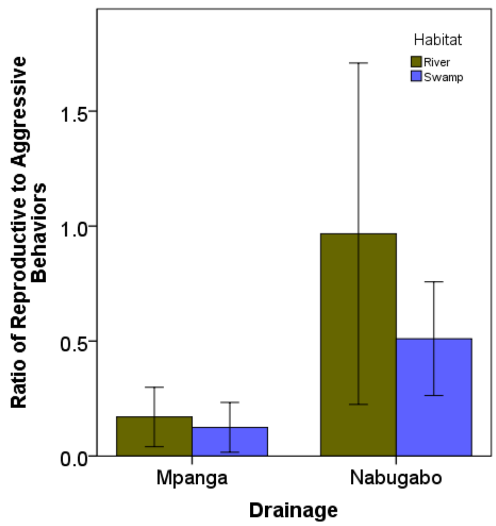
Reproductive Behaviors	Aggressive Behaviors
lateral display frontal display lead quiver	chasing biting

Figures

Figure 1: Location of two study systems, Mpanga and Nabugabo, in Uganda, Africa. *P. multicolor* are found in rivers and swamps of both drainages.

Figure 2. A) Male and **B)** Female *Pseudocrenilabrus multicolor victoriae*

A)



B)

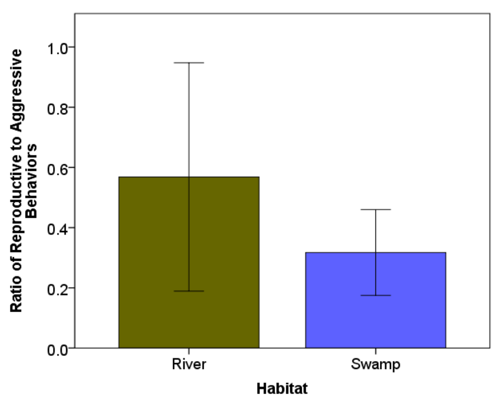


Figure 3: Mean ratio (± se) of reproductive to aggressive behaviors observed in (A) two river and two swamp populations from two different drainages, and (B) for river and swamp habitats (populations pooled). Values are shown are non-transformed but transformed (log) values were used for analyses.